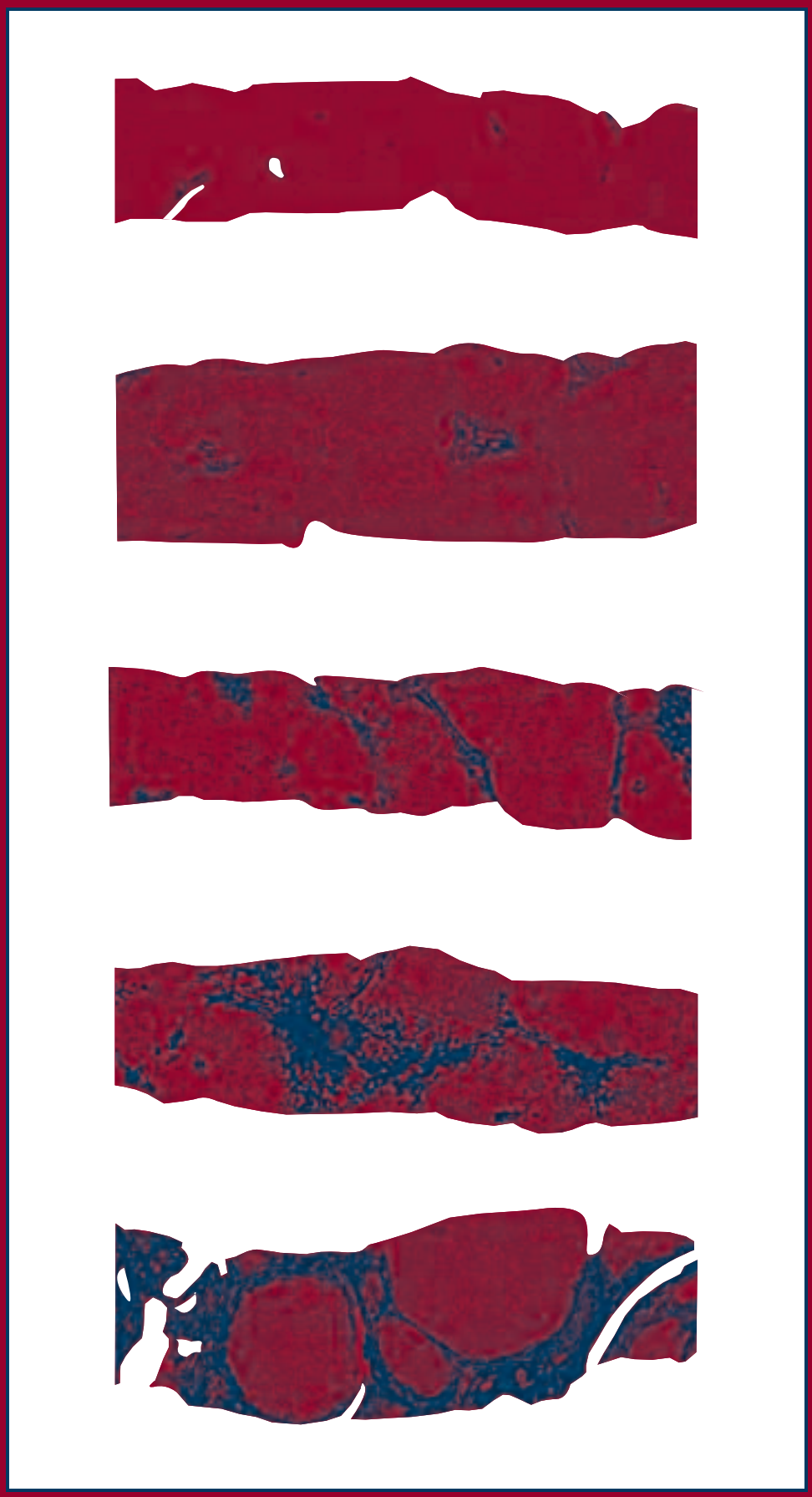

Liver fibrosis is a disease process that occurs in response to injury and involves the formation of scar tissue composed mainly of the protein collagen. In liver biopsies, such as those shown, which were taken from five patients with varying degrees of liver fibrosis, collagen is visualized in blue using a special stain. From top to bottom, these biopsies show the progressive stages of fibrosis from small amounts of collagen deposition to a severe form of fibrosis called cirrhosis. Photo: Dr. Zachary Goodman, Division of Hepatic Pathology, Armed Forces Institute of Pathology.

CHAPTER 2: LIVER INJURY, INFLAMMATION, REPAIR, AND FIBROSIS

INTRODUCTION AND BACKGROUND

Tissue injury and inflammation, repair, and fibrosis are fundamental components of acute and chronic liver diseases regardless of cause. Hepatocellular injury and cell death, with the accompanying inflammatory response, provoke symptoms of fatigue and weakness, and account for elevations of serum aminotransferases that are characteristic of liver disease. More importantly, hepatic injury and inflammation also lead to loss of liver function. The ensuing responses of cell repair, regeneration, and fibrosis ultimately determine whether patients recover from liver disease or develop progressive scarring of the liver, which can result in cirrhosis and portal hypertension.

Clarifying the cellular and molecular mechanisms that underlie these processes is critical for designing and developing effective treatments for patients with liver disease. In some instances, therapies can be targeted against the specific agents that cause liver disease, such as the hepatitis B and C viruses. In most liver diseases, however, therapy against intermediate processes mediating liver injury would greatly benefit patients. Such therapies would include means to decrease cell injury and inflammation, promote regeneration and repair, and ameliorate fibrosis. Because the mechanisms of injury and repair are similar among a variety of liver diseases, therapies directed against these pathways are likely to be helpful for a wide range of conditions. Drugs that prevent liver cell death, inhibit liver fibrosis, and promote repair will emerge only from a detailed understanding of these processes, including the complex, interwoven pathways involved. New therapies that result from careful research into the mechanisms of liver cell death, hepatic inflammation, fibrosis, and repair could have an immense salutary impact on human health and disease, both by saving lives and by substantially reducing health care expenditures.

RECENT RESEARCH ADVANCES

The last decade has witnessed tremendous progress toward uncovering the fundamental mechanisms that contribute to liver injury, inflammation, repair, and fibrosis. Despite this progress, new insights have not been sufficiently translated into innovative therapies. Unlike breakthroughs in other major biomedical fields, such as atherosclerosis and cancer, advances in understanding liver cell biology have not yet had a meaningful impact on the natural history or prevalence of liver disease. Progress to date must be extended to uncover new tools for basic discoveries and opportunities for translational advances.

Insights into Liver Injury and Repair Pathways:

Examples of recent research advances in this area include solid evidence demonstrating that: apoptotic cell injury is generic to many liver diseases; sinusoidal cells are critical for hepatocyte survival; hepatic stellate cells/myofibroblasts are a key source of collagen deposition in liver fibrosis; and hepatic fibrosis has a

reversible component. An understanding of progenitor cell biology and the role of these cells in tissue repair has also emerged. Critical intracellular signaling cascades culminating in apoptotic cell death, inflammatory cell recruitment, and collagen production have been elucidated. The extracellular matrix has been found to play an important role in cell signaling and the repair process. Disease-specific and cell type-specific responses have become apparent, which may allow some therapeutic targeting to these pathophysiologic events.

Knowledge Gaps in Understanding Liver Injury and Repair: Despite these advances, substantive gaps in knowledge persist and more progress could be pursued through additional and expanded research initiatives. Although several ligand/receptor-initiated intracellular signaling cascades and gene expression profiles have been characterized, we know little about which pathways are dominant in specific liver cell populations *in vivo*, how these pathways interact functionally, and what impact they have on expression and persistence of disease. Even more striking is the lack of knowledge about the innate and adaptive immune response to liver injury from viruses or metabolic and autoimmune diseases.

Obvious questions persist in these and other areas, such as: How do the innate and adaptive immune systems react to and modulate apoptosis, liver repair, hepatic metabolism, and fibrosis? Can deleterious aspects of metabolism, including its resultant generation of reactive nitrogen and oxygen species, be modified to minimize disease progression? How do apoptosis and oncotic necrosis influence fibrosis? What is the basis for the remarkable property of the injured liver to regenerate, which distinguishes it from all other solid organs? What are the source(s) and fates of progenitor cells in liver injury? How can specific liver toxicities be predicted and prevented? What are the genetic determinants of liver disease, and how does the interplay between genes and environment influence the expression of liver disease?

There are also important deficiencies in the tools available to address these questions. For example, the field has been hampered by a lack of definitive and relevant disease models, which limits the translation of scientific information into therapeutic opportunities. Databases of genetic information focused on liver disease have yet to be developed, and tools to apply genetic information to the discovery of disease mechanisms are lacking. Finally, studies performed in cellular models require extension and implementation in whole organ and animal models.

RESEARCH GOALS

The major research goals in liver injury, inflammation, repair, and fibrosis are to understand the cellular mechanisms mediating these processes and to develop effective means for monitoring and treating the diseases caused by these processes.

Pathophysiology of Liver Injury: Insights into liver injury will require information regarding the intra- and inter-cellular responses to toxic stimuli. Liver damage is frequently characterized by cell type-specific injury. For example, hepatocytes are the primary target of attack in viral hepatitis, cholangiocytes in primary biliary cirrhosis, and endothelial cells in sinusoidal obstruction syndrome (veno-occlusive disease). Major gaps remain in knowledge of the fundamental responses of specific liver cell populations to injury and how cell injury leads to inflammation.

- *Research Goals:* To identify cell type-specific cytotoxic signaling pathways in the liver and to determine how liver cells produce and respond to inflammatory mediators (Matrix Cells A1 and B1).

Ideally, these cell-specific pathways would be correlated with changes in genomic and proteomic patterns of expression, with care to include the analysis of post-translational modification of proteins by glycosylation, phosphorylation and oxidative or nitrosative processes.

In addition to provoking cell type-specific responses, many liver disease processes affect more than one hepatic cell type, thus eliciting complex intercellular responses. For example, activated resident macrophages known as Kupffer cells can generate reactive oxygen species and cytokines, which in turn injure adjacent parenchymal cells. Also, inflammatory stimuli can induce enzymes that generate nitric oxide, leading to nitrosative stress. Perturbations of intrahepatic blood flow commonly accompany clinical liver disease and are especially germane to the organ preservation injury that can occur in the setting of liver transplantation.

- *Research Goal:* To understand these intercellular responses and their consequences, future research might focus on the integrative mechanisms mediating oxidative, nitrosative, hypoxic, and ischemia-reperfusion injury. Certain cell types in the liver, such as endothelial and Kupffer cells, also play important roles in these processes and deserve careful assessment (Matrix Cell B2).

Liver injury also provokes infiltration and adhesion of circulating leukocyte populations into the liver, which can modify and amplify the injury response.

- *Research Goal:* To further elucidate the impact of leukocyte sub-populations (e.g., neutrophils, cytotoxic T lymphocytes, natural killer cells, macrophages) and their mediators on liver injury, fibrosis, and regeneration (Matrix Cell A2).

New discoveries in this area will likely yield novel therapeutic targets and approaches.

Small Molecules as Therapeutic Agents: Small molecules have proven useful as therapeutic agents in a wide range of human afflictions. The pharmaceutical and biotechnology industries are often sources of such agents; however, opportunities to develop promising therapeutics for liver disease have not been uniformly pursued.

- *Research Goal:* To develop high-throughput screens to identify candidate small molecules capable of modifying cytotoxic and fibrotic pathways and of regulating the repair response in liver cells (Matrix Cell C2).

A key research objective is for investigators to partner with industry whenever possible in developing high-throughput screens relevant to liver disease. Such efforts could provide cost-effective opportunities for pursuing selected compounds as therapies in preclinical studies.

Preclinical Studies of Liver Cell Injury and Fibrosis Pathways: Although reductionist, cell-based experimental systems are vital for elucidating signaling pathways that culminate in liver cell injury and fibrosis, the complex, integrated processes occurring in disease can only be fully evaluated in animal models. Furthermore, therapeutic endpoints can only be rigorously assessed *in vivo*.

- *Research Goal:* To develop relevant and robust animal models that faithfully mimic the development and resolution of human hepatic injury and fibrosis (Matrix Cell C1).
- *Research Goal:* To evaluate how nutritional factors affect liver cytotoxic, injury-response, and fibrotic pathways (Matrix Cell A1).

Clinical Research in Liver Injury, Inflammation, Repair, and Fibrosis: The ultimate goal of research in this area is to translate findings in experimental liver injury, inflammation, repair, and fibrosis into meaning-

ful advances in diagnosing, monitoring and treating human liver disease. Toward this goal, better biomarkers for liver disease progression and response to therapy would be helpful.

- *Research Goal:* To analyze proteomic changes in the liver and patterns of liver-derived serum proteins in specific diseases, which may allow earlier diagnosis and more specific therapeutic intervention (Matrix Cell B2).

Ultimately, such information could obviate the need for liver biopsy, an invasive procedure that is currently a mainstay of liver disease evaluation. Also, because industry is reluctant to fund clinical studies that require invasive clinical endpoints that can impact morbidity and mortality, the development and validation of biomarkers to assess ongoing treatment efficacy would greatly stimulate clinical research in liver disease.

- *Research Goal:* To complement biomarker development, determine the genetic determinants of disease risk and progression in acute and chronic liver injury, fibrosis, and regeneration (Matrix Cell C2).

Identification of genetic determinants would refine clinical trial enrollment and shorten treatment intervals by stratifying patients according to risk, allowing for earlier evidence of efficacy. Genetic risk information would also facilitate the transition from preclinical studies to human trials.

Focused and efficient translation of new findings in liver injury, inflammation, repair, and fibrosis will depend upon the coordinated development of clinical trials of these new therapies, such as anti-apoptotic and hepatoprotective therapy for viral hepatitis, nonalcoholic steatohepatitis, or drug-induced liver injury.

- *Research Goal:* To define the role of nonspecific, anti-apoptotic therapy in liver disease (Matrix Cell A2).
- *Research Goal:* To develop gene-, cell-, or pharmacology-based therapy for hepatic injury (Matrix Cell B3; see also Chapter 8, C2).

More challenging is the development of an antifibrotic therapy, an area where long-term studies with careful analysis of hepatic fibrosis and cirrhosis would be helpful.

- *Research Goal:* To develop a pharmacotherapy that limits hepatic fibrosis through targeting pathways that promote and terminate fibrosis (Matrix Cell C3).

Definitive, phase III trials of such therapies should only be undertaken after phase I and II trials demonstrate preliminary safety, tolerability, optimal-dose regimen, and suitability of surrogate markers of effect.

Elucidation of the pathways of collagen formation, deposition, and resolution would help to identify potential candidate markers that might be identified in serum or urine. Use of a noninvasive biomarker rather than liver biopsy to assess development of fibrosis and its resolution would greatly aid the development of therapeutics directed against the fibrotic process.

- *Research Goal:* To develop noninvasive markers for fibrogenesis and the amount of fibrosis (Matrix Cell A3; see also: Chapter 6, C2; Chapter 7, B2; Chapter 16, C1).

STEPS TO ACHIEVE RESEARCH GOALS

Multifaceted, complementary strategies are necessary to achieve these research goals in the areas of liver injury, inflammation, repair, and fibrosis.

An emphasis on investigator-initiated studies would optimally address many of the goals related to patho-

physiology. However, to identify potential small molecule therapies for liver disease, larger scale efforts will be required that are supported independently of a hypothesis-driven funding format. Such efforts will require mechanisms to encourage industry to partner with academia and invest in liver disease research, while overcoming issues of intellectual property and other legal barriers. The model provided by the National Cancer Institute provides one potential template to surmount these problems.

The development of animal models, although resource-intensive, is nonetheless essential to furthering advances in the field. A well funded consortium dedicated to developing relevant and robust animal models of human liver diseases is necessary to achieve this goal.

Finally, human studies will require the integration of clinical research networks with investigators versed in proteomics, genomics, and molecular medicine. Major emphasis could be given to funding grant applications that focus on the rapid translation of new research findings into innovative, but practical, approaches to therapy of liver disease.

All of these vital goals are achievable with a clear and sustained focus and adequate resources.

Matrix of Research Goals in Liver Injury, Inflammation, Repair, and Fibrosis

	Short Term (0-3 years)	Intermediate Term (4-6 years)	Long Term (7-10 years)
High Risk	**A3.** Develop noninvasive biomarkers for fibrosis.	**B3.** Develop gene-, cell-, or pharmacology-based therapies for hepatic injury.	**C3.** Develop mechanism-based drug therapy in fibrotic disease, targeting pro-fibrogenic and fibrosis resolution pathways.
Intermediate Risk	**A2.** Define the role of antiapoptotic therapy in liver injury, fibrosis, and regeneration. Identify the impact of individual leukocyte sub-populations and their mediators on liver injury, fibrosis, and regeneration.	**B2.** Identify the integrative mechanisms mediating oxidative, nitrosative, hypoxic, and ischemic-reperfusion injury and the role of sinusoidal cells. Identify the proteomic response of the liver and liver-derived serum proteins as intermediate biomarkers for liver disease progression and response to therapy.	**C2.** Using high-throughput screens, identify candidate small molecules that modify cytotoxic and fibrotic pathways in liver cells. Define genetic determinants of disease risk and progression in acute/chronic liver injury, fibrosis, and regeneration.
Low Risk	**A1.** Identify individual liver cell type-specific responses to inflammatory mediators. Elucidate whether and how nutritional factors affect liver cytotoxic and fibrotic pathways.	**B1.** Identify individual liver cell type specific extrinsic (e.g., mediator-based) and intrinsic (e.g., organelle-based) cytotoxic signaling pathways.	**C1.** Develop relevant and robust animal models of hepatic injury, inflammation, and fibrosis progression and resolution.